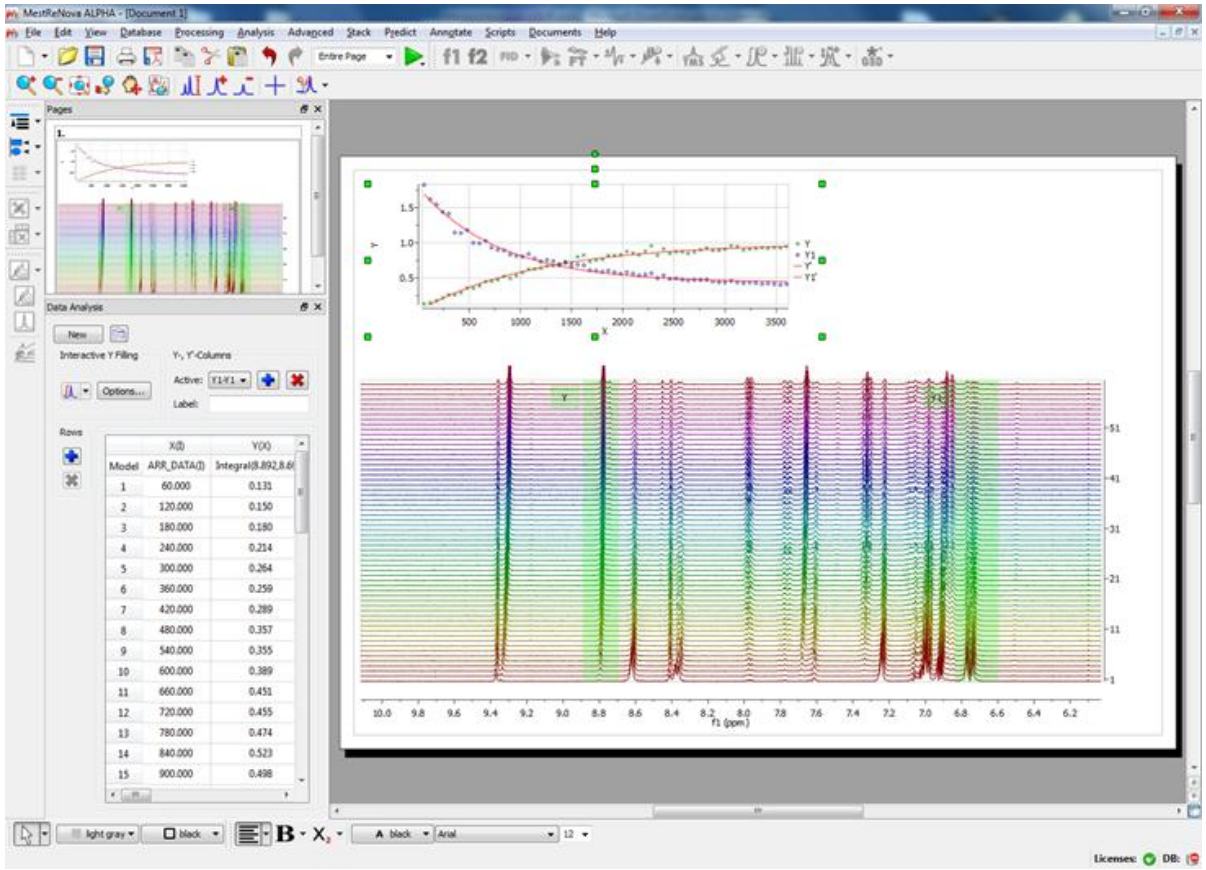
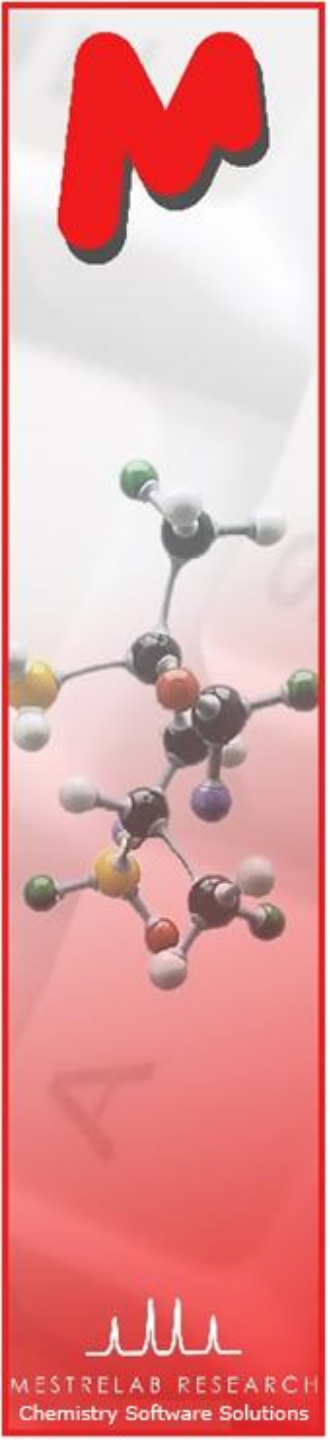
Mnova 8 for Reaction Monitoring by NMR



Version 8.0
Aug. 2012

Chen Peng, PhD
VP of Business Development, US & China
Mestrelab Research SL
San Diego, CA
(858) 736-4563
chen.peng@mestrelab.com

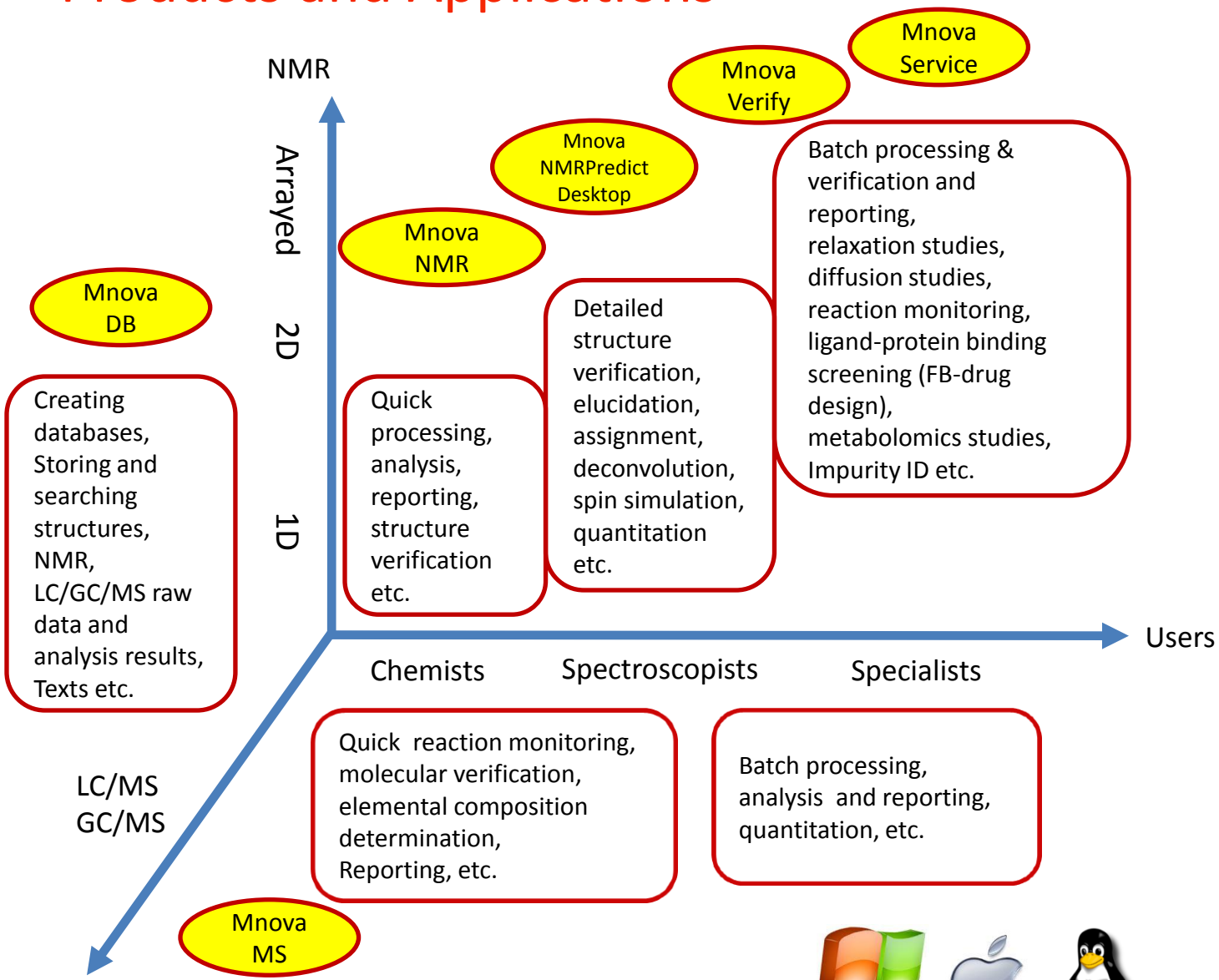
MESTRELAB RESEARCH
Chemistry Software Solutions



Outline

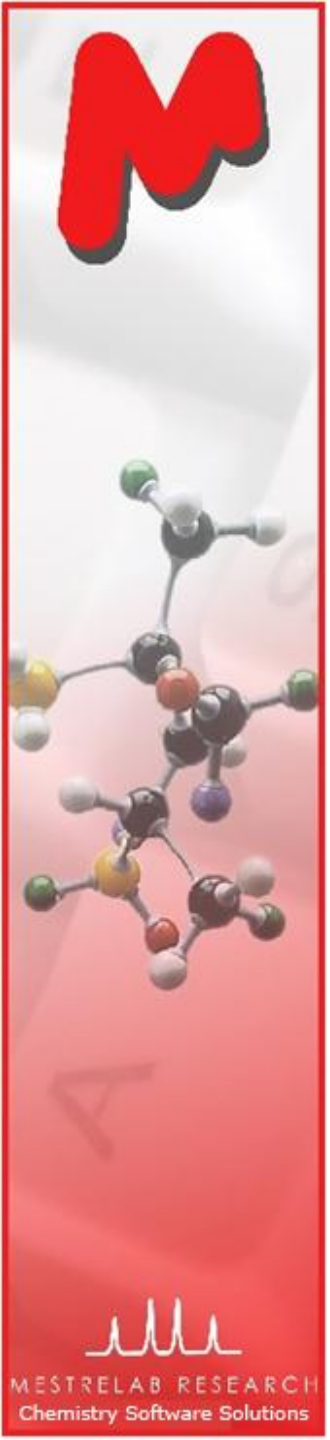
- About Mestrelab Research
- Importing and processing arrayed NMR spectra
- Extracting spectral information
- Fitting spectral data to a kinetics function
- Summary

Products and Applications



Mnova is compatible with Mac, Windows and Linux

MESTRELAB RESEARCH
Chemistry Software Solutions



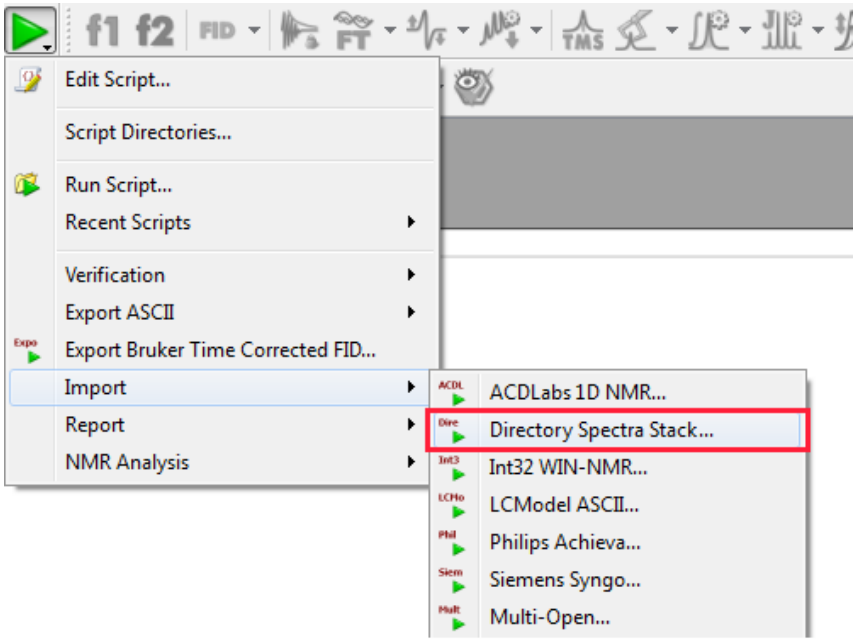
Mestrelab Research

- 1996: A research project in University of Santiago de Compostela, Spain, developed free **MestReC** software for NMR processing
- 2004: **Mestrelab Research** incorporated in Santiago de Compostela
- 2004: New **MestreNova (Mnova)** platform and **NMR** plugin released
- 2006: **NMRPredict Desktop** plugin released with Modgraph
- 2009: **LC/GC/MS** plugin released with Sierra Analytics
- 2009: Global Spectral Deconvolution (**GSD**) algorithm released with ExtraByte
- 2011: **DB** plugin for Database Management
- 2012: **Verify** plugin for auto structure verification and peak assignment
- 2012: **qNMR** and **Screen** plugins - to be released
- An **R&D company** with >20 people and >80,000 registered users

www.mestrelab.com

To open and process reaction monitoring data

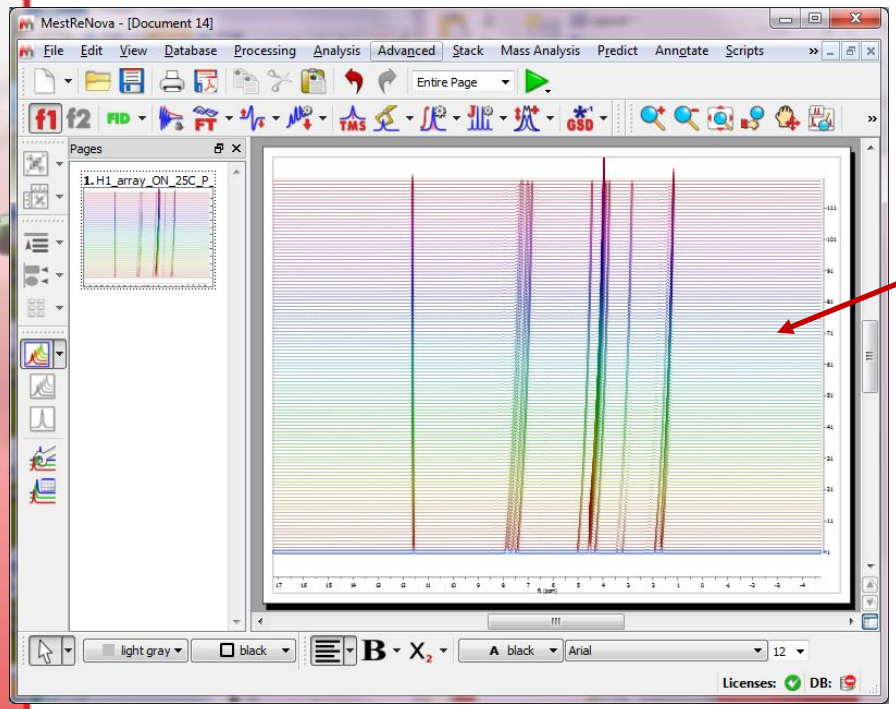
- In a typical kinetics or reaction monitoring (RM) experiment, a series of spectra are recorded at predetermined time intervals to follow the progress of the reaction:
  - All spectra are acquired and stored as *individual spectra*
  - All spectra are acquired and stored as a single NMR experiment in *arrayed mode*
- Mnova supports both kinds of data
- For RM data acquired on individual basis: Run the **Directory Spectra Stack** script to open and stack all spectra under a base directory:

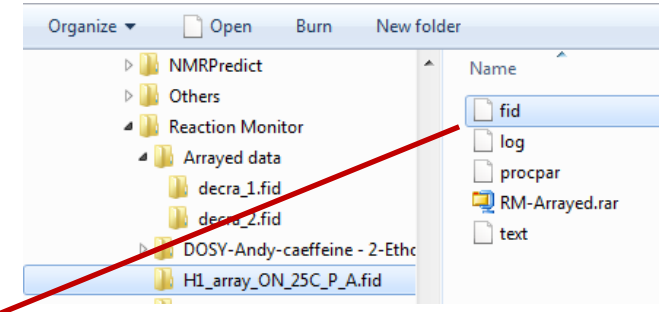


- *The user selects the directory where the spectra are located*
- *Mnova opens and process all those spectra (FIDs) automatically using the processing parameters from the instrument*
- *Mnova stacks all spectra in the alphabetic order of the folder name of the experiments (You can change the order later – see subsequent slides)*

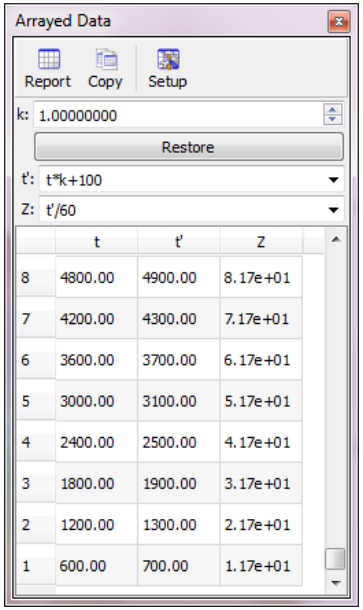
To open and process reaction monitoring data

- For RM data acquired in arrayed mode: Just open the FID (Varian) or SER file (Bruker), and the individual spectra will be processed and stacked.
  - Mnova parses the arrayed parameters automatically and displays them in the Arrayed Data Table (Choose **View | Tables | Arrayed Data** to open it)





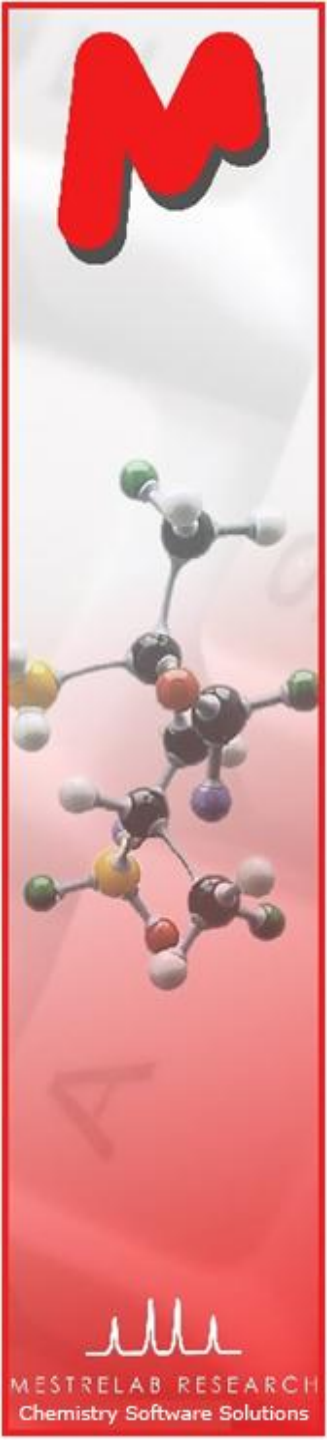
Drag & drop



	t	t'	Z
8	4800.00	4900.00	8.17e+01
7	4200.00	4300.00	7.17e+01
6	3600.00	3700.00	6.17e+01
5	3000.00	3100.00	5.17e+01
4	2400.00	2500.00	4.17e+01
3	1800.00	1900.00	3.17e+01
2	1200.00	1300.00	2.17e+01
1	600.00	700.00	1.17e+01

t is the arrayed parameter. Use factor *k* and define formulas to convert it into reaction times (*Z*) for subsequent analysis.

MESTRELAB RESEARCH
Chemistry Software Solutions

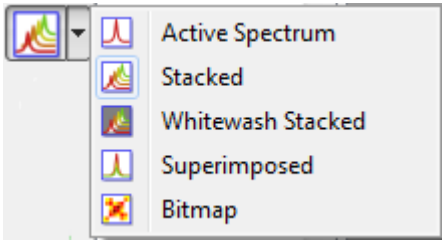


Easy handling multiple spectra in Mnova

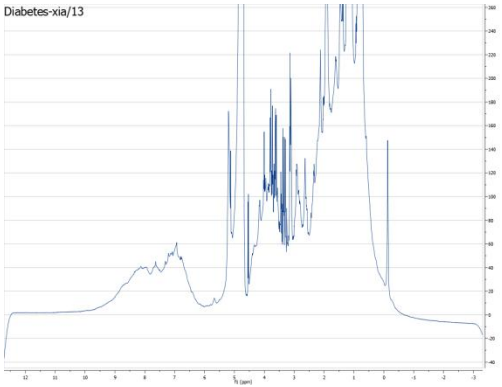
- With Mnova, it is very easy to
  - To display stacked spectra in different modes, such as Stacked, Superimposed, Active Spectrum, or Bitmap
  - To re-process all the spectra together, such as phasing and baseline correction (this is the default)
  - To select one or several spectra and apply processing only to them
  - To hide any number of spectra for better visualization
  - To correct global or local spectral misalignment
  - To normalize the Y-scale of the spectra in various ways

To change the stacking mode

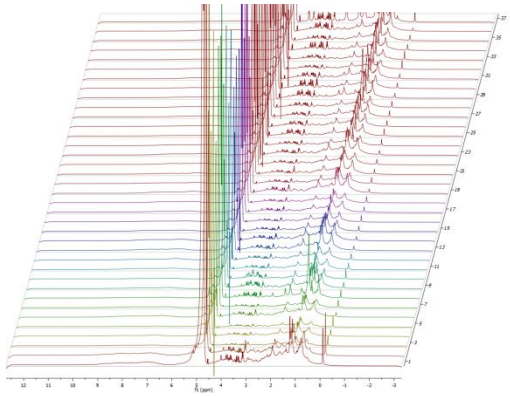
- Click  to choose the display mode for stacked spectra



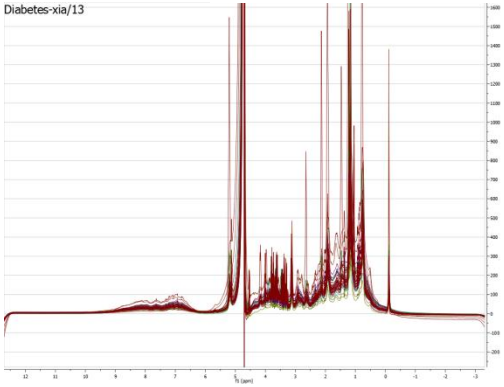
Active Spectrum



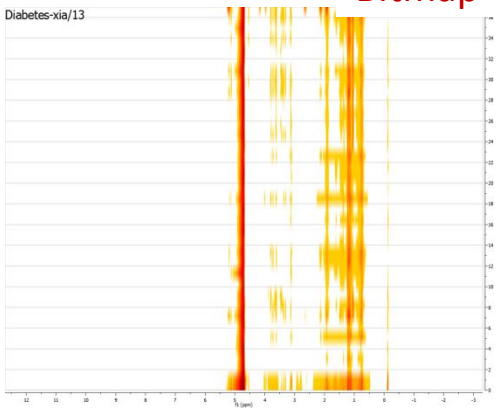
Stacked



Superimposed



Bitmap

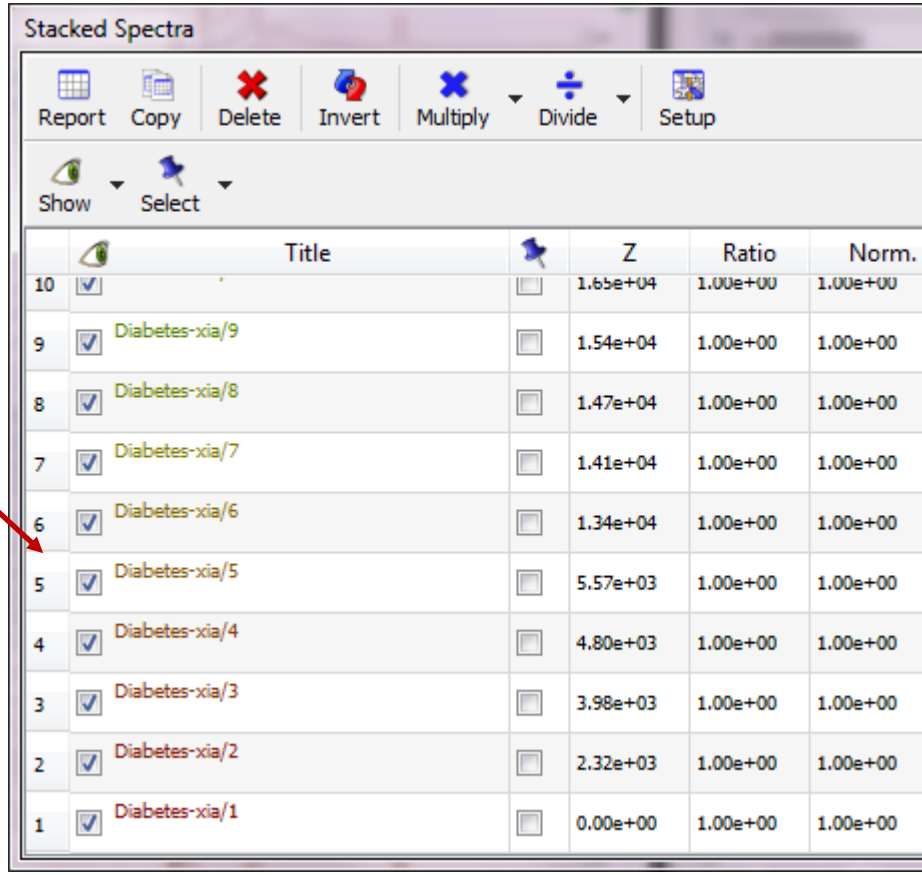


MESTRELAB RESEARCH
Chemistry Software Solutions

To re-process the stacked spectra

- Click  to toggle on the **Stacked Spectra Table**
- Use this table to do the following:
  - Delete spectra from the stack
  - Change order of the spectra in the stack
  - Change the Y-intensity of selected spectra
  - Change which ones to display
  - Change which ones to re-process, such as phasing, baseline correction etc.

Click and drag here to change the order of a spectrum in the stack



Tip: The Titles of the spectra can be customized by double-clicking on the stacked spectra, and changing the Title-related settings in the Properties dialog. For arrayed spectra, use "Spectrum Number" as the title or part of the title.

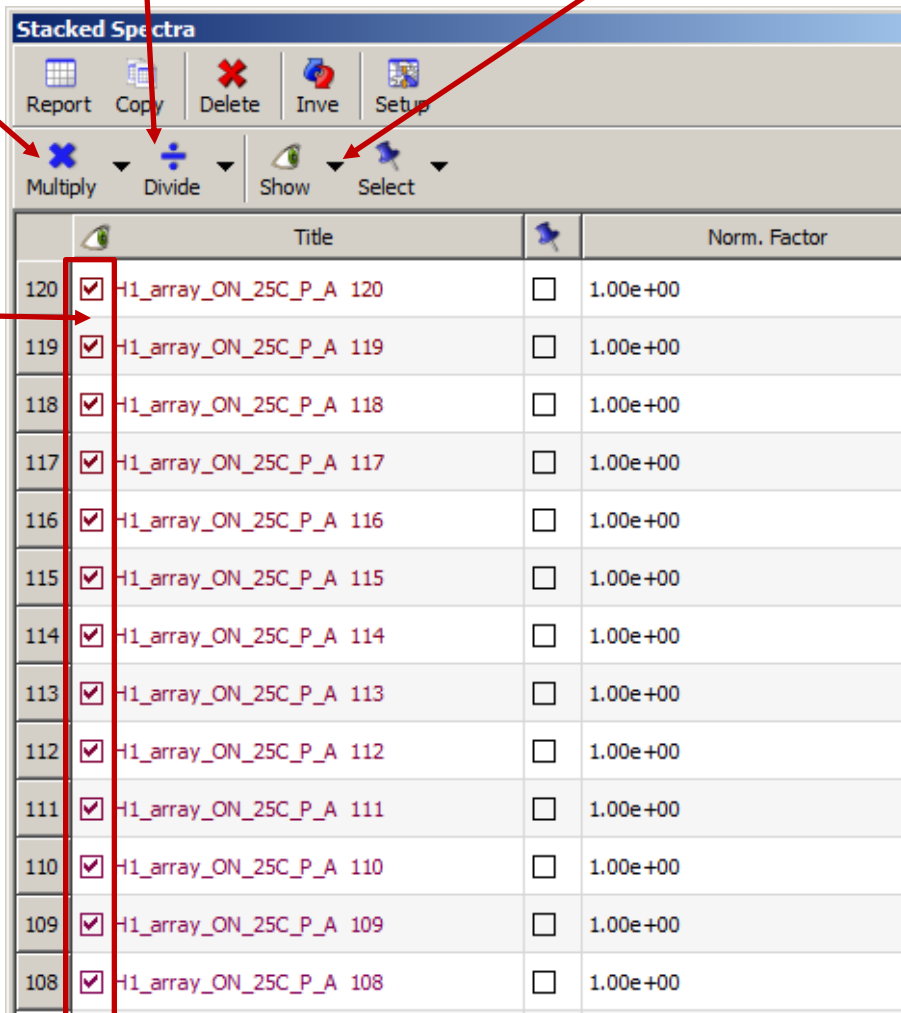
Use Stacked Spectra Table to setup the display

To increase the Y intensity of selected or all spectra *

To decrease Y intensity of selected or all spectra*

Click the arrow here for more options for spectral display

Un-check a spectrum if you don't want to show it in the stack



Note these commands change the Y intensity values of the spectrum/spectra. They are mainly used for visualization of spectra with very different intensities. Do* ***not* *use them for quantitative analysis.*

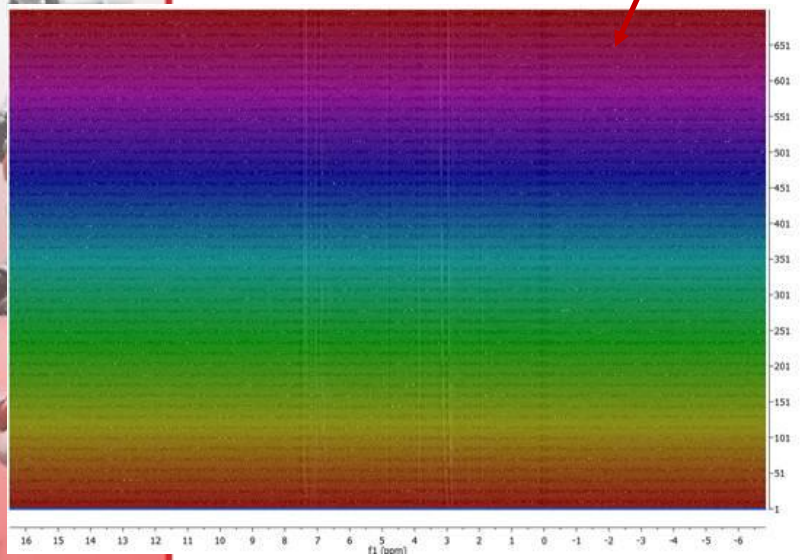
Tip: Click Setup to change the display options for this Stacked Spectra Table

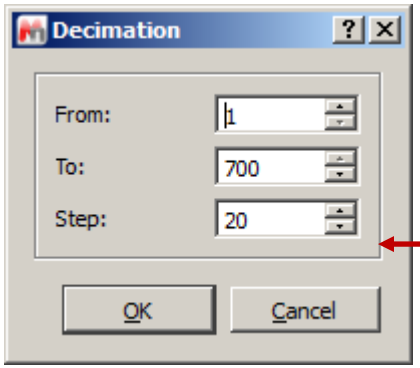
MESTRELAB RESEARCH
Chemistry Software Solutions

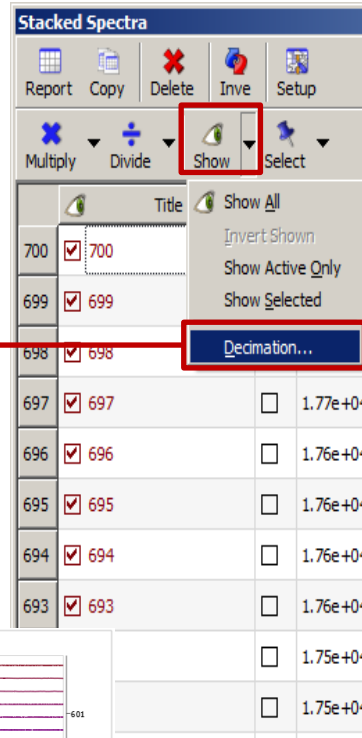
Use Stacked Spectra Table to setup the display

- Use the **Decimation** option when the number of stacked spectra is very large:

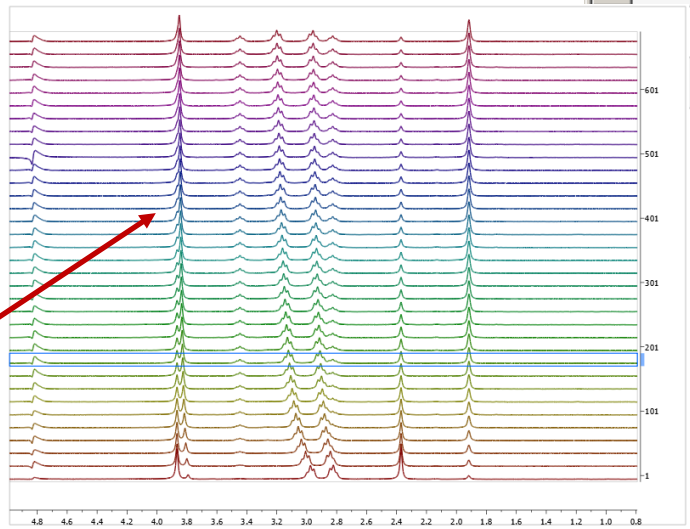
700 spectra all displayed







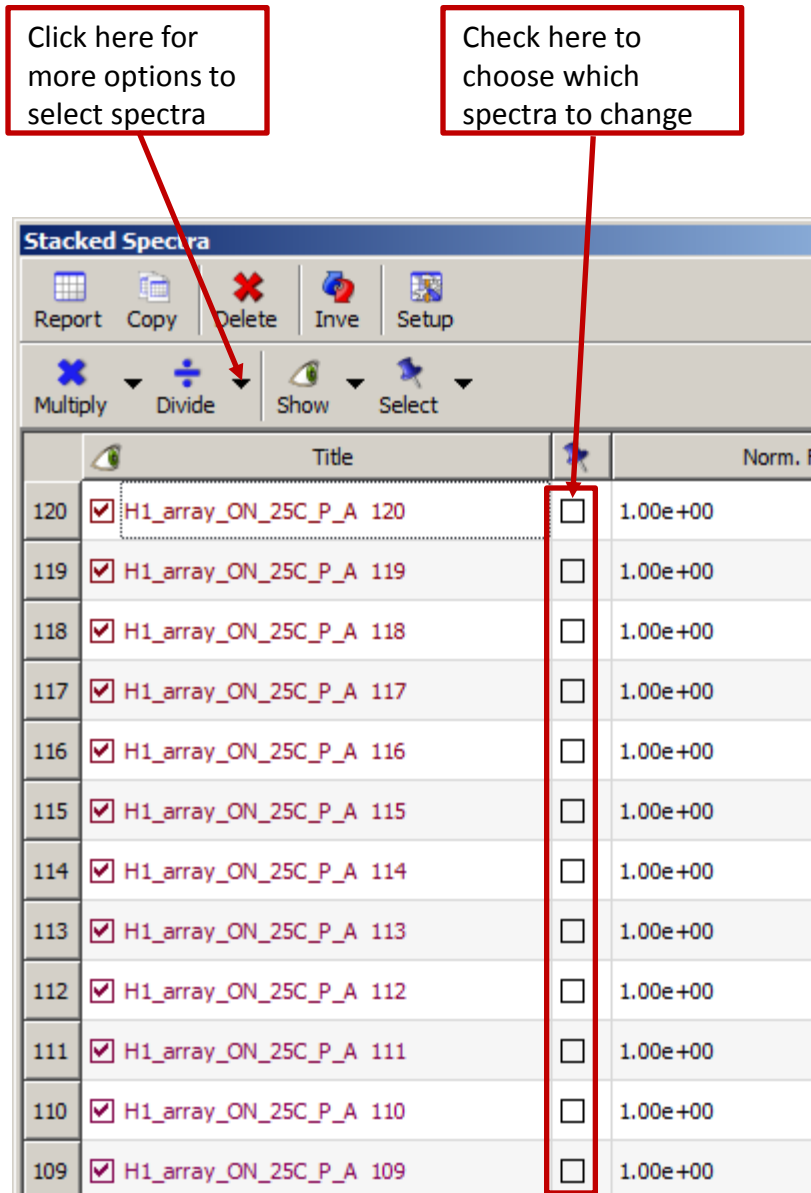
Only one spectrum displayed for every 20 spectra



MESTRELAB RESEARCH
Chemistry Software Solutions

To re-process *all* or *selected* spectra

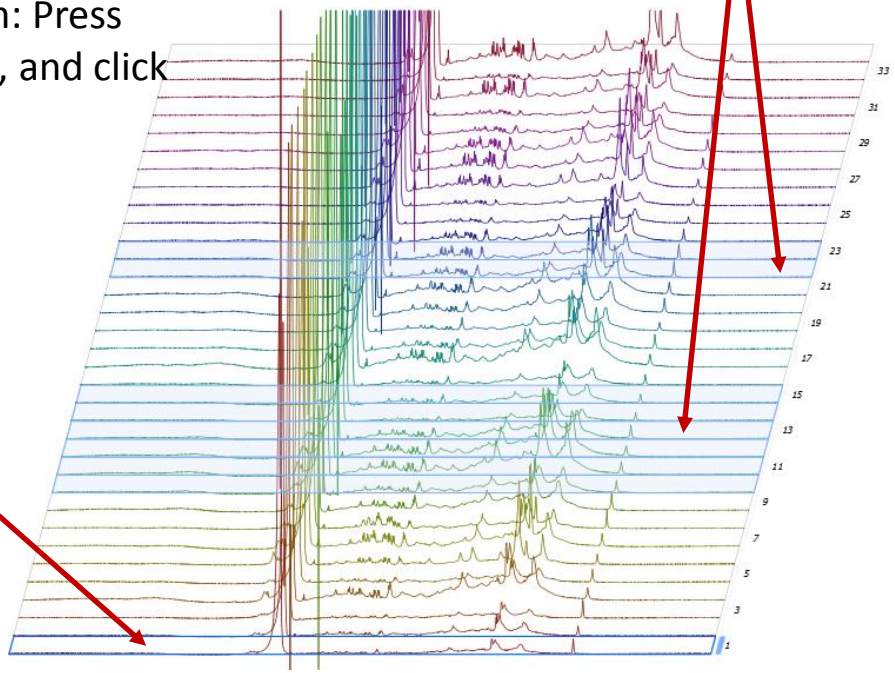
- Spectra are automatically processed when they are opened, but sometimes you need to manually re-process all or some of them
- Which ones will be changed when you apply the processing tools?
  - If no spectrum is checked in the Select column, **all** spectra will be changed
  - If some spectra are checked in the Select column, only the **selected** ones will be changed
- Use **Undo/Redo** if you've made a mistake



To select spectra from the stacked spectra

- Spectra can also be selected from the spectral stack directly.
- To select one spectrum: Press and hold **Alt** key, and click on the spectrum
- To select multiple spectra: Press and hold **Ctrl** or **Shift** key, and click on a spectrum
- To de-select one spectrum: Press and hold **Ctrl** and **Alt** keys, and click on the spectrum

Selected spectra: processing will apply on them only

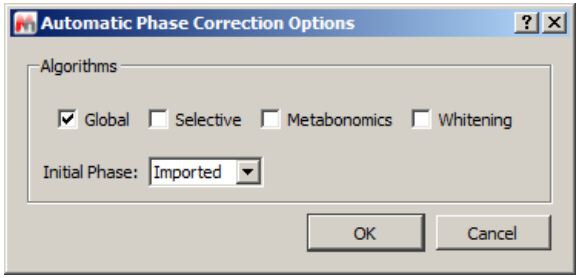


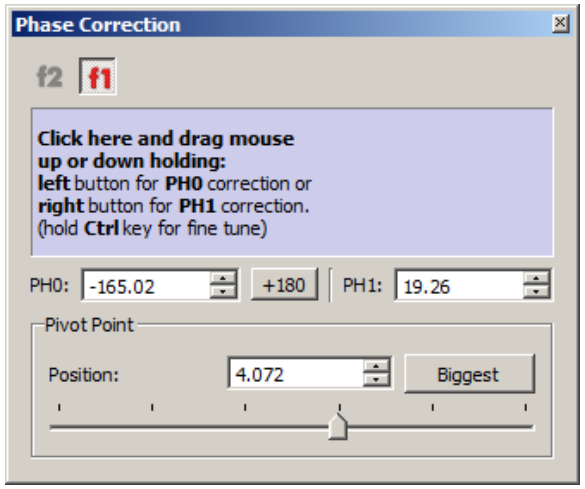
The active spectrum: It will be displayed when in *Active spectrum* mode. It is also used as reference spectrum in some operations such as spectral alignment

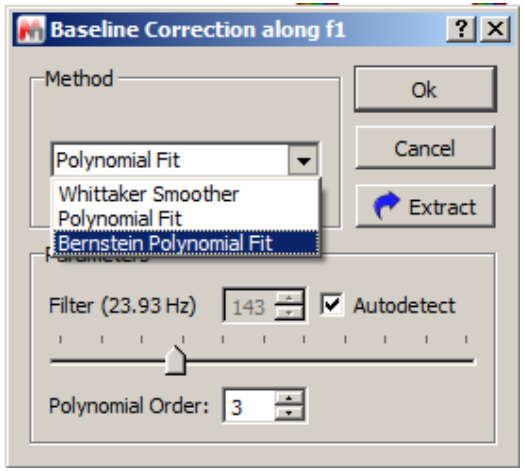
MESTRELAB RESEARCH
Chemistry Software Solutions

To correct phase errors and baseline

- Click  for **phase correction** if peaks are not symmetric. Options:
  - Global method for all positive peaks
  - Selective method for positive/negative peaks
  - Metabolomics method when there is residual solvent peaks
  - Whitening method by maximizing the baseline regions
  - You can combine any of the methods listed above
  - Manual method if none of the above works
- Click  for **baseline correction** if baseline is not zero. Options:
  - Polynomial Fit
  - Bernstein Polynomial Fit
  - Whittaker Smoother
  - Manual



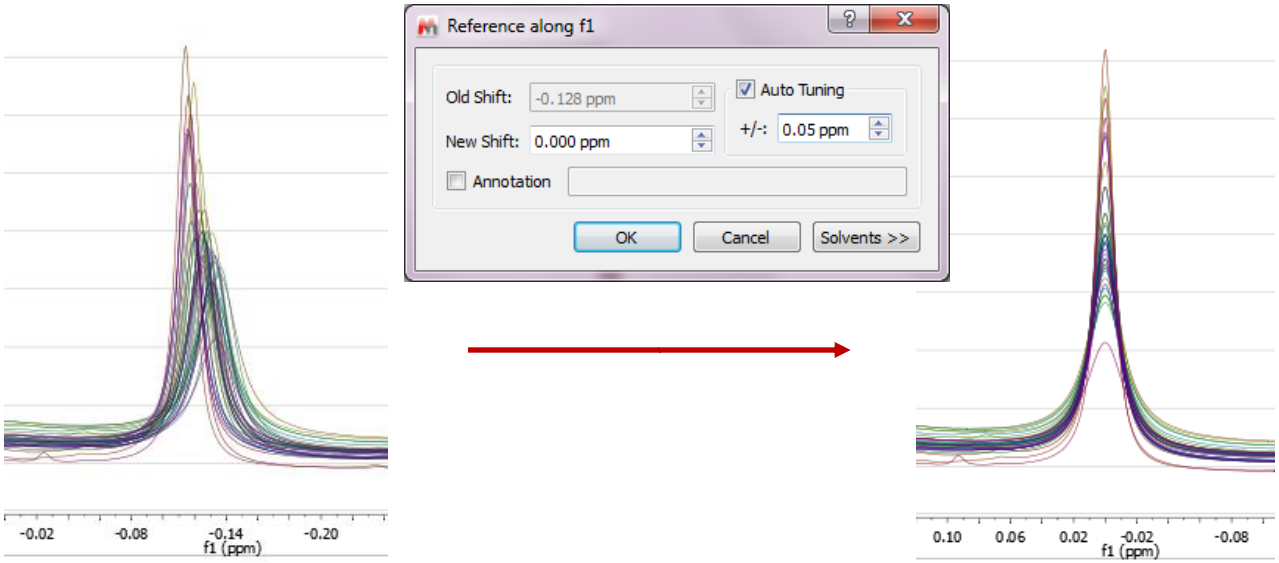




MESTRELAB RESEARCH
Chemistry Software Solutions

To align spectra by correcting reference

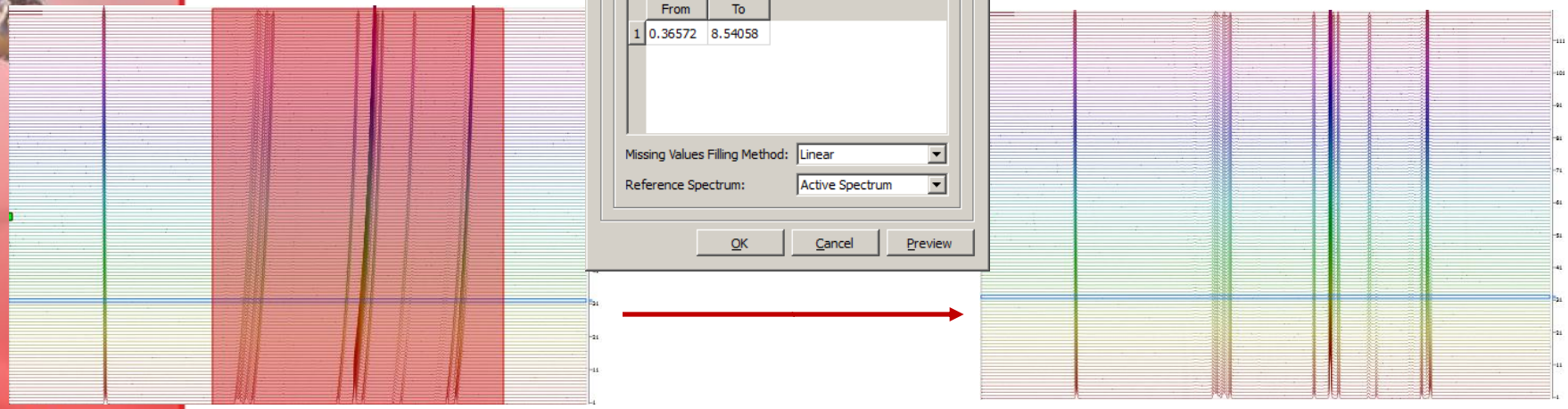
- Systematic errors of chemical shift reference can be corrected if there is an internal reference peak, e.g. TMS peak.
- Click  and then click on the reference peak in the active spectrum
- In the following dialog, set the proper chemical shift for the reference peak, check Auto Tune, and define a tuning region (e.g. +/- 0.05 ppm) *:



**Note: it is important to make sure that the reference peak is always the highest one within the tuning region for all spectra*

To correct local peak misalignment

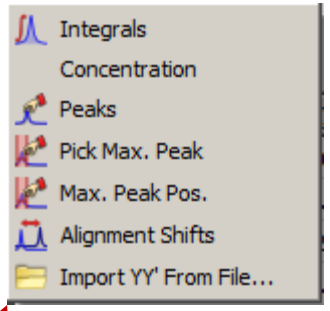
- Zoom into the region of interest, select **Advanced | Align Spectra**.
- Click  , then click-and-drag to cover the peaks to align. Click Preview to see the alignment result. Adjust other parameters until satisfactory.
- Move to other regions to continue this process until done.
- Click OK to accept the results



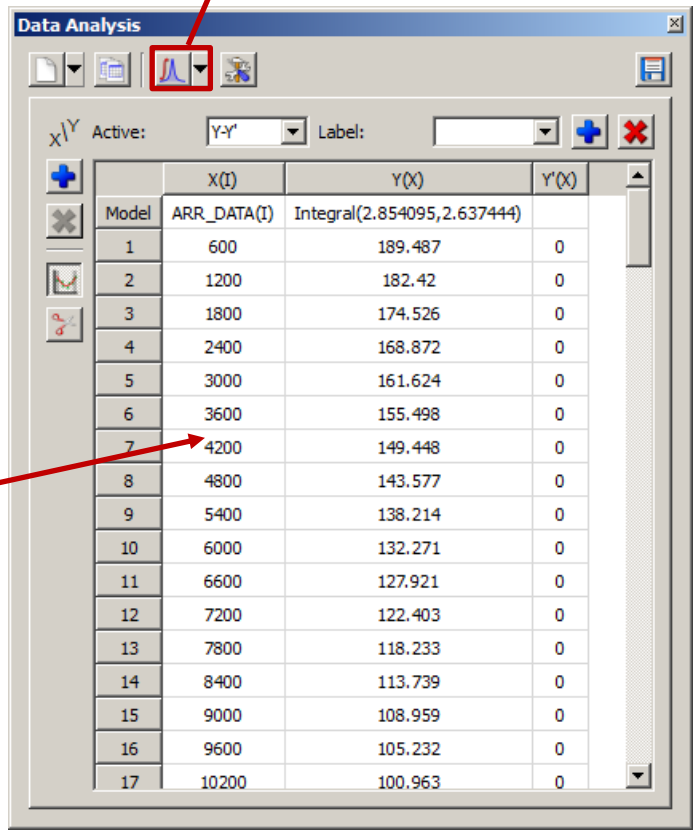
Tip: When there is peak cross-over, it may not be good idea to use local peak alignment. Instead, use the UI feature to change the integration regions so that they follow the change of the peak locations. See subsequent slides.

To analyze stacked spectra using the Data Analysis Panel

- The **Data Analysis Panel** provides an intuitive way to analyze multiple stacked spectral data
- Choose **View | Panels | Data Analysis** to open the Data Analysis Panel
- Click **Create Empty Graph** Tool  to create a new analysis.
- Choose one of the **data extraction modes** (e.g. Integrals), click and drag in the spectra to define the range for extracting such information (e.g. integrating the peaks).



The X(I) column is automatically filled with the reaction time. Use Arrayed Data Table to preview and convert those data.
You can also manually edit these data, or copy from a .txt file.

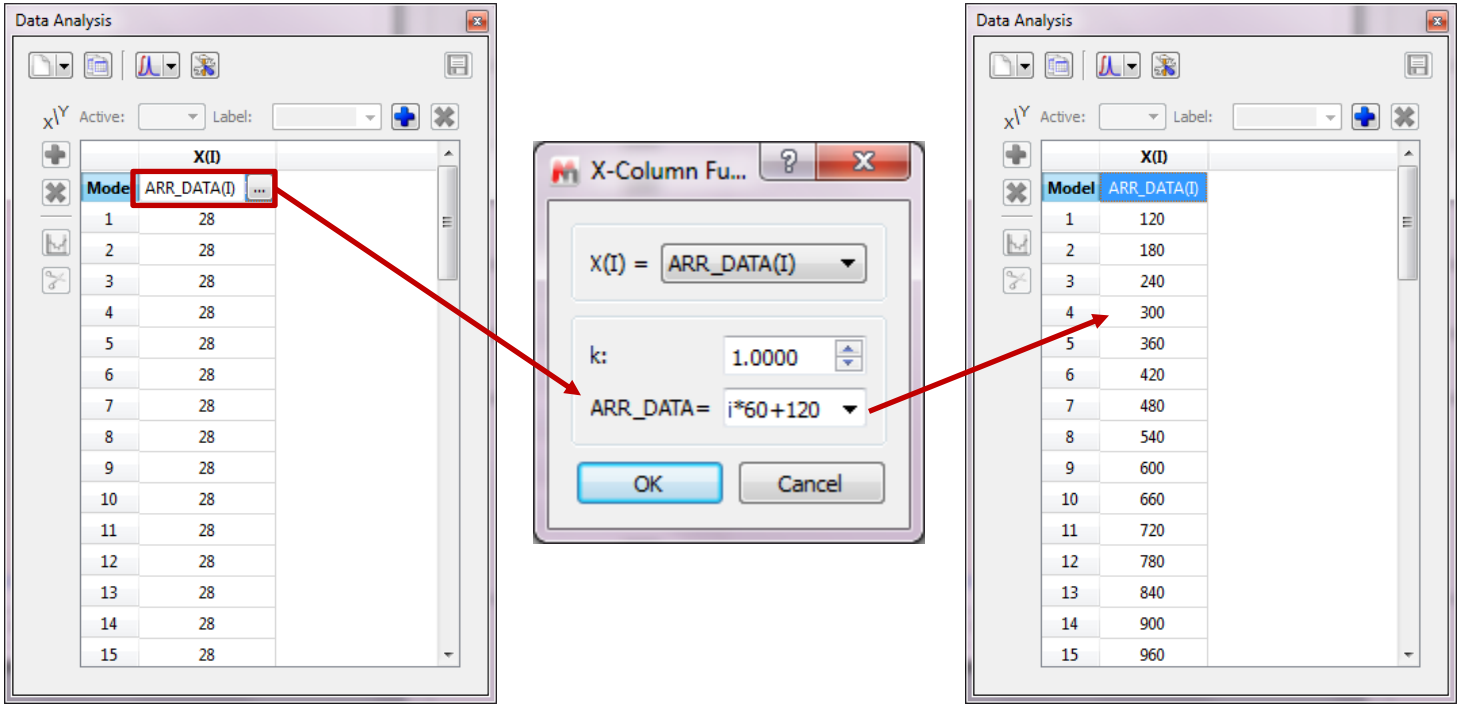


	X(I)	Y(X)	Y'(X)
Model	ARR_DATA(I)	Integral(2.854095,2.637444)	
1	600	189.487	0
2	1200	182.42	0
3	1800	174.526	0
4	2400	168.872	0
5	3000	161.624	0
6	3600	155.498	0
7	4200	149.448	0
8	4800	143.577	0
9	5400	138.214	0
10	6000	132.271	0
11	6600	127.921	0
12	7200	122.403	0
13	7800	118.233	0
14	8400	113.739	0
15	9000	108.959	0
16	9600	105.232	0
17	10200	100.963	0

MESTRELAB RESEARCH
Chemistry Software Solutions

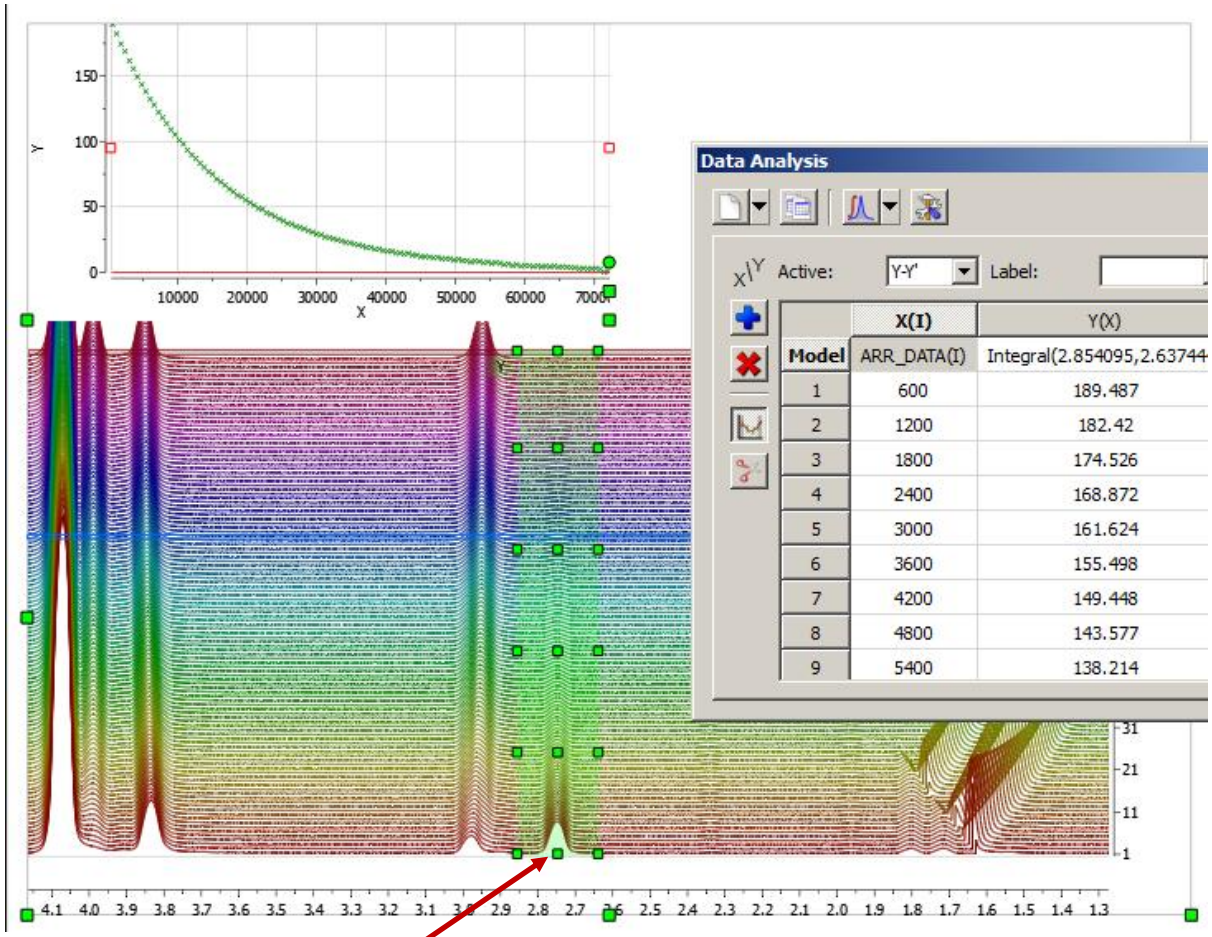
What if reaction time is not properly read?

- Normally the reaction times are automatically read to **Arrayed Data Table** (see previous slides) and automatically populated to X(I) column here
- Occasionally the reaction times are not properly read to Arrayed Data Table, and hence not displayed correctly in Data Analysis Panel
- In such a case, click ARR_DATA(I) and the "…" button, define a function of ***i*** (spectral index) to calculate the reaction times if you can
- Or enter the reaction times manually. You can copy such numbers from a text file and paste to the table



To extract data using the Data Analysis Panel

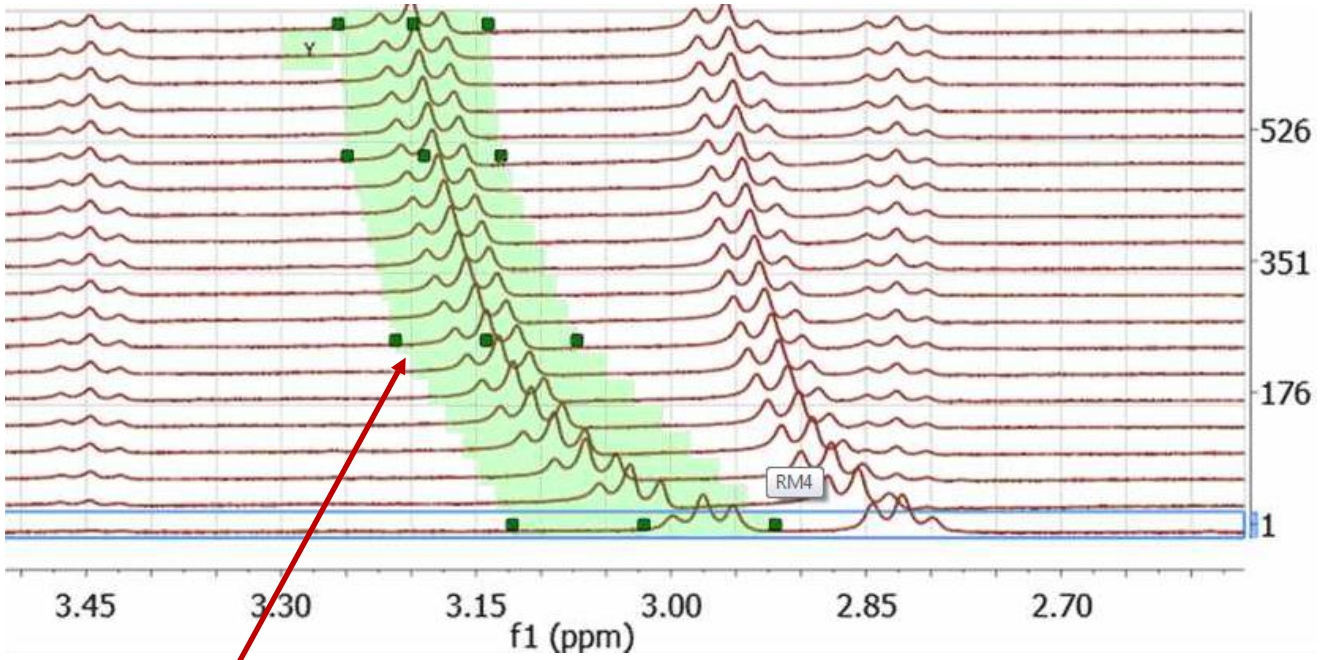
- If **Integrals** is chosen (the default setting), the integrals within the defined region are filled in the Y(X) column, and also plotted in the X-Y graph (X = reaction time, Y= integrals).



The region within which peaks are integrated as Y(X) values

To extract data from drifting peaks

- If the peaks drift over time, you can manually change the direction of the integration regions :



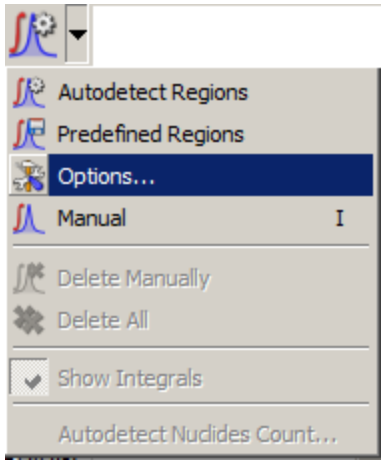
Click & drag the handles to change the shape of the selection region.
Press Shift to move all points simultaneously

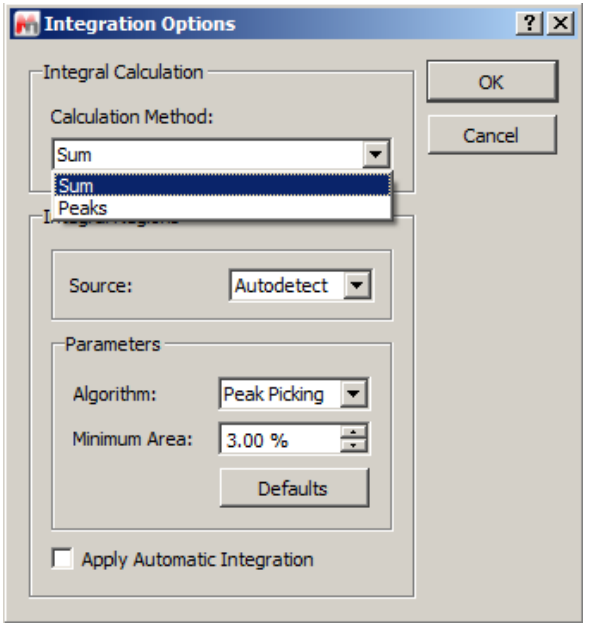
Tip: you can change the number of handles by clicking the Options button on the Data Analysis Panel: 

MESTRELAB RESEARCH
Chemistry Software Solutions

More options for peak integration: Sum, GSD/LSD, or Line-fitting methods

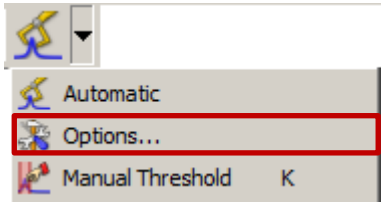
- By default, the integration is done by summing up all points within the defined region. This is the default setting – **Sum Method**.
- When peaks are partially overlapping, it is useful to use the **Peaks Method** so that only the deconvolved peaks are integrated.
- The is controlled by the **Integration Options**

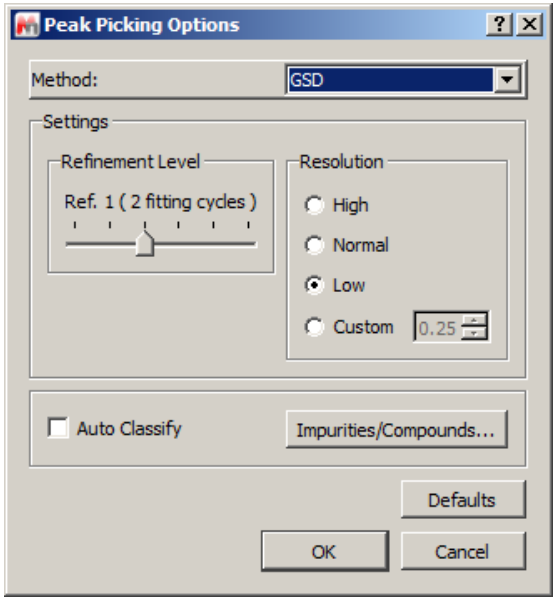




Peak integration using GSD (Global Spectral Deconvolution)

- To use the Peaks-based integration, you can do the following:
  - Do GSD to all the spectra first, and then define the integration region(s), or
  - Do integration directly to the spectra from the Data Analysis Panel: This will do Local Spectral Deconvolution (LSD) to all the spectra within the region*
- You can also use the Line Fitting tools to refine the GSD peaks locally.
- When an integration region is defined from Data Analysis Panel, the deconvolved peaks whose tops fall within the region are summed up as the integral for that spectrum



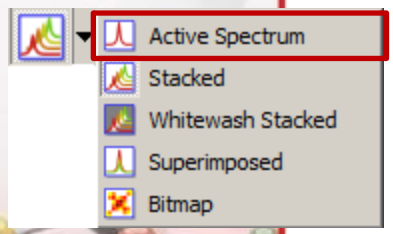


Tip: deconvolution of multiple spectra can be very slow, and unreliable if the peaks are very small. So use it with caution. You are recommended to save the document before such operations.
** LSD can be faster than GSD, but you cannot change the integration region to outside of the current one(s) later, as Mnova will not re-do the LSD if you change the current integration region*

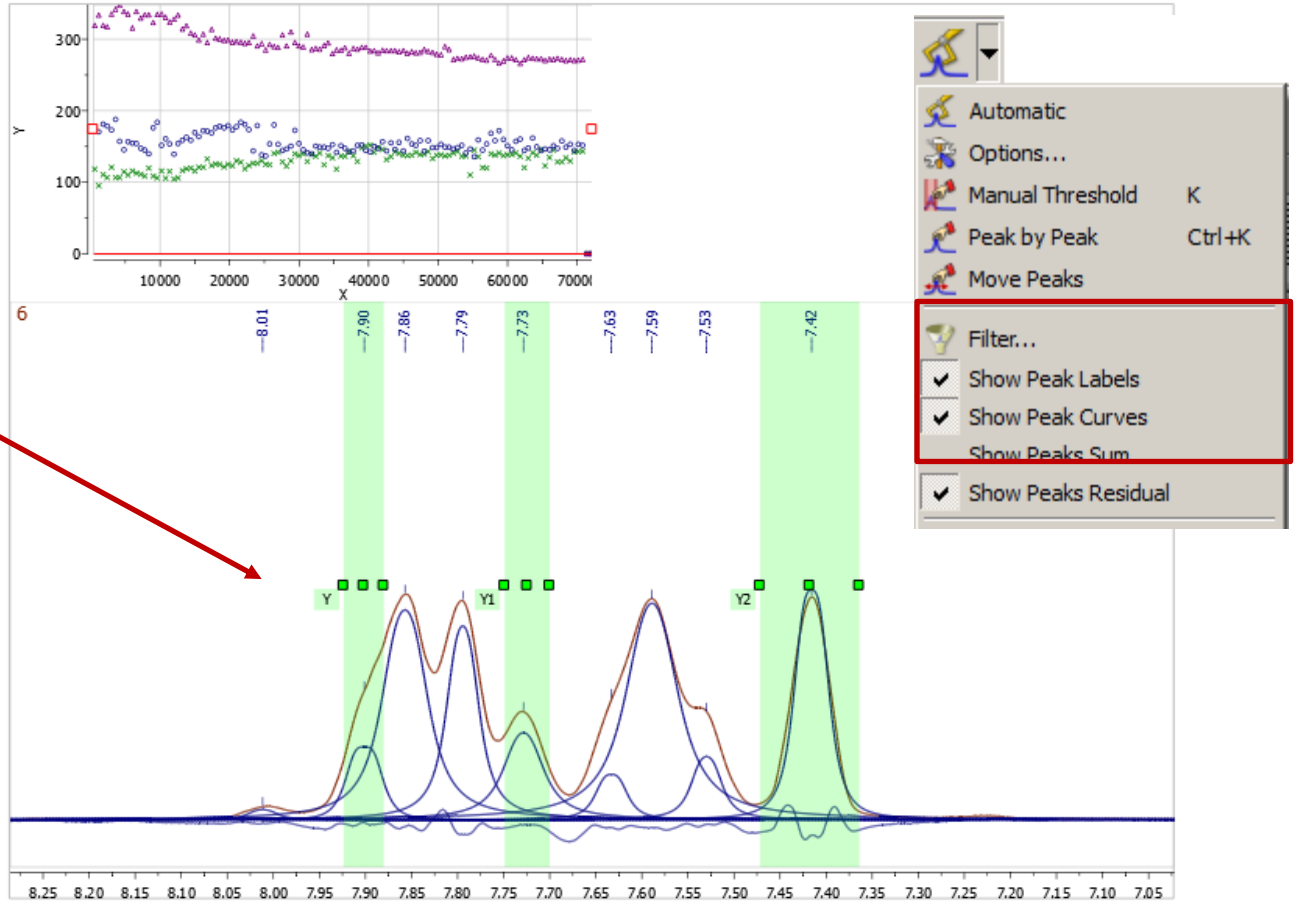
MESTRELAB RESEARCH
Chemistry Software Solutions

Peak integration using GSD (Global Spectral Deconvolution)

- In this example, LSD is first done within 8.2 – 7.2 ppm by defining an integration region of 8.2-7.2 ppm
- Next the Integration region is shrunk to cover only the peak around 7.9 ppm, and two more regions are added to cover the other two interesting peaks (7.73 and 7.41 ppm). The details are examined as the following:



Set to **Active Spectrum mode**, and turn on the **Peak Curves** and **Residual Curves** to see the details of peak deconvolution for each spectrum. Use Shift+ Arrow Keys to browse through the stacked spectra

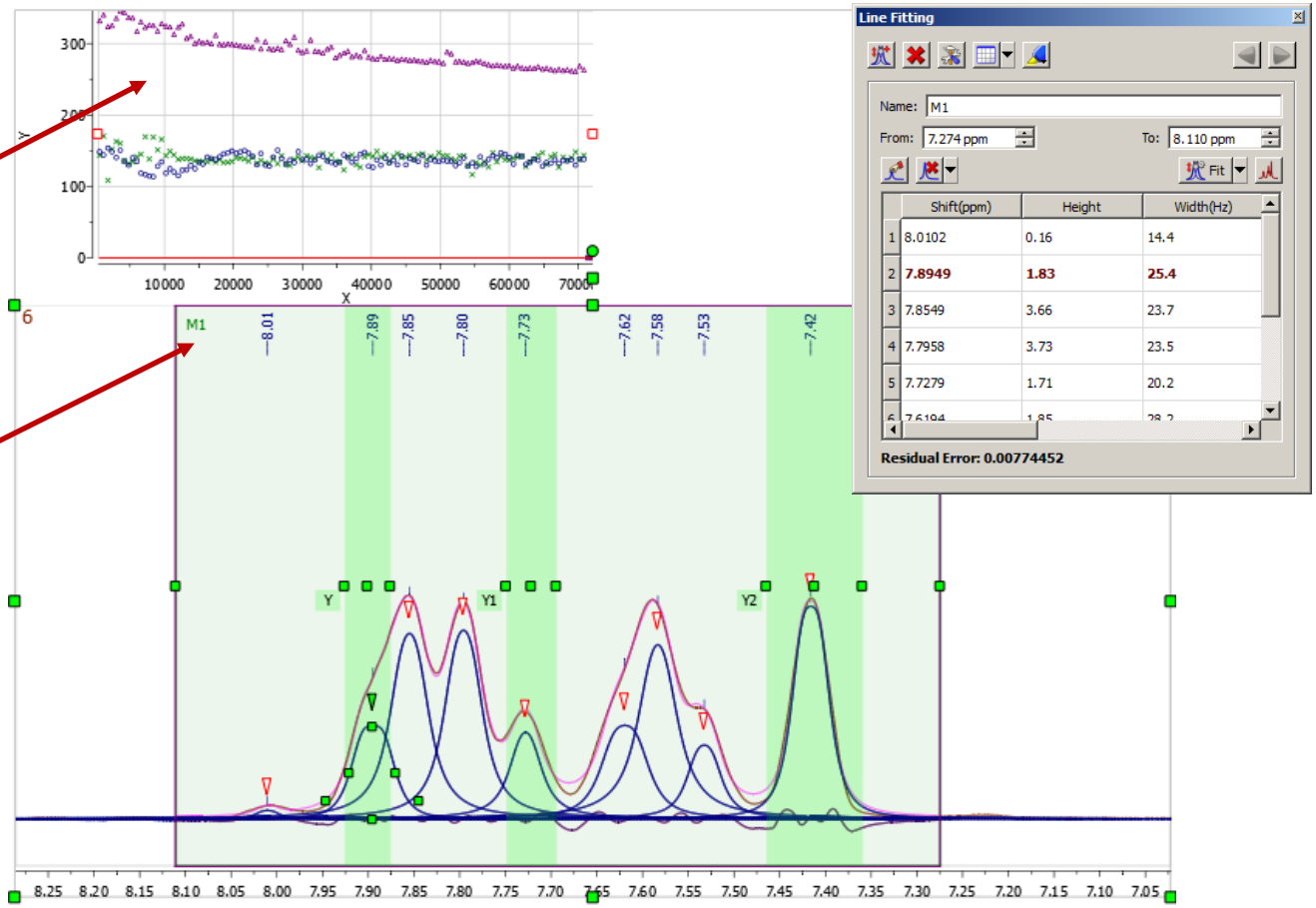


Refining GSD/LSD results using Line Fitting

- Optionally, we use the **Line Fitting Tools** to define a region that covers all the GSD/LSD peaks
- Click the **Fit** button and the line fitting is done for all spectra in the same region to optimize the GSD peaks and achieve better fitting
- Finally we shift the integration regions slightly to update the integrals based on the optimized GSD peaks

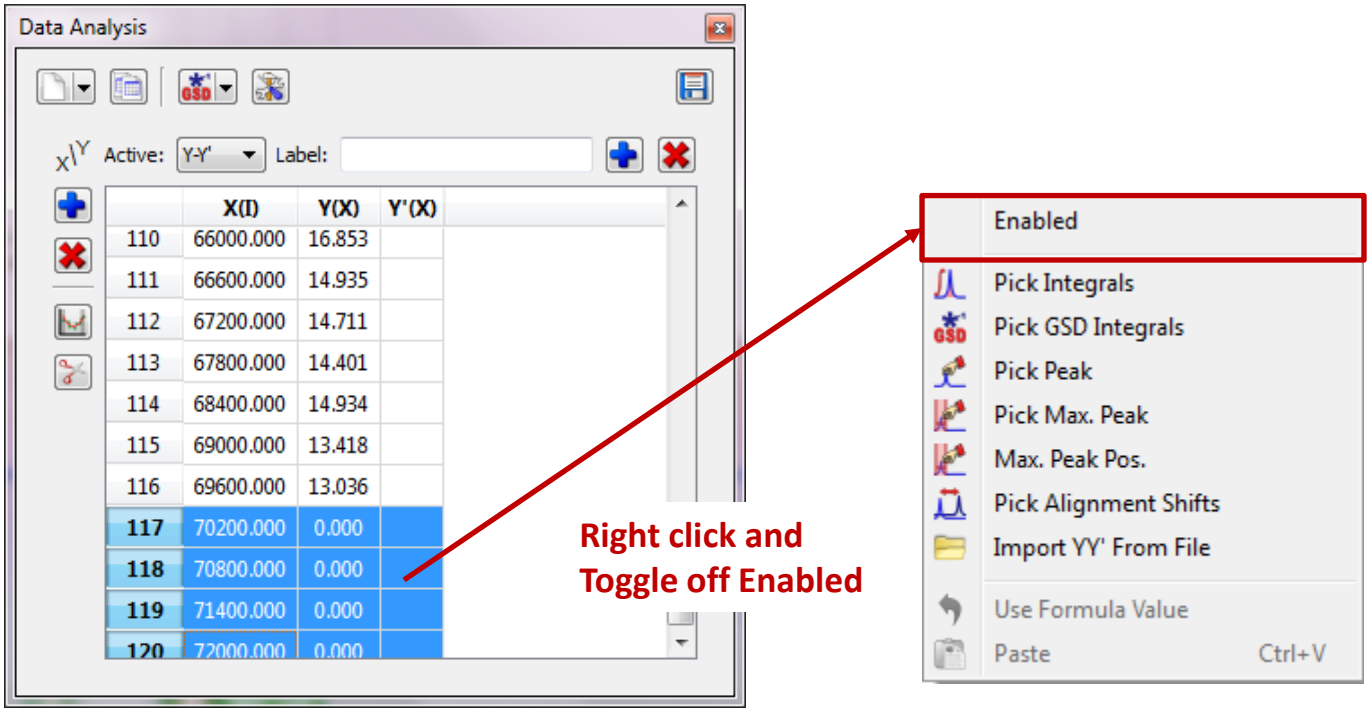
X-Y points after refining the GSD peaks using Line Fitting

Line fitting region. All GSD peaks are used as starting points, and are systematically varied to achieve a better fit (smaller residual) to the spectral curve for all spectra



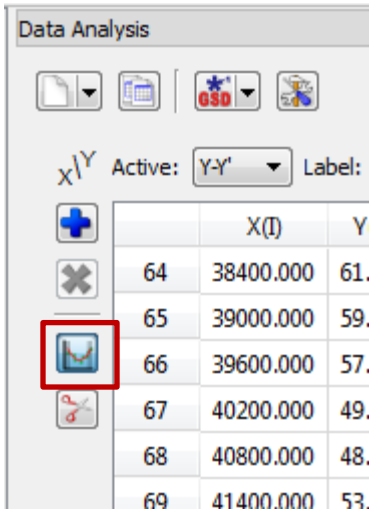
What to do with bad points?

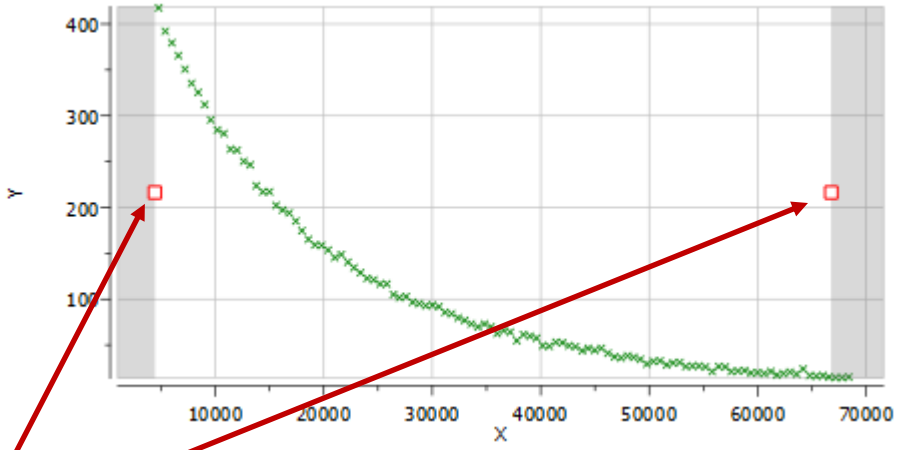
- To exclude some data points, highlight them in the table, and right click and turn off Enabled:



What to do with bad points?

- To exclude some data points at the beginning or at the end of the reaction, you can toggle on the **Use Fitting Limit** button, and exclude the data points on the XY Graph:



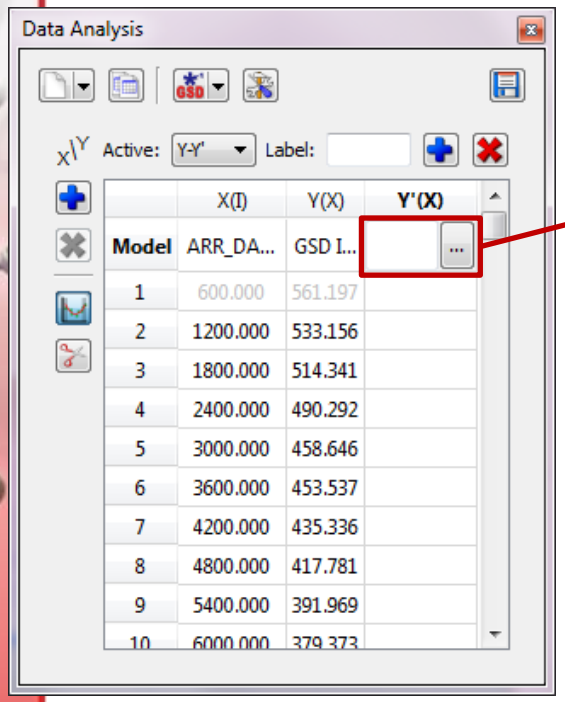


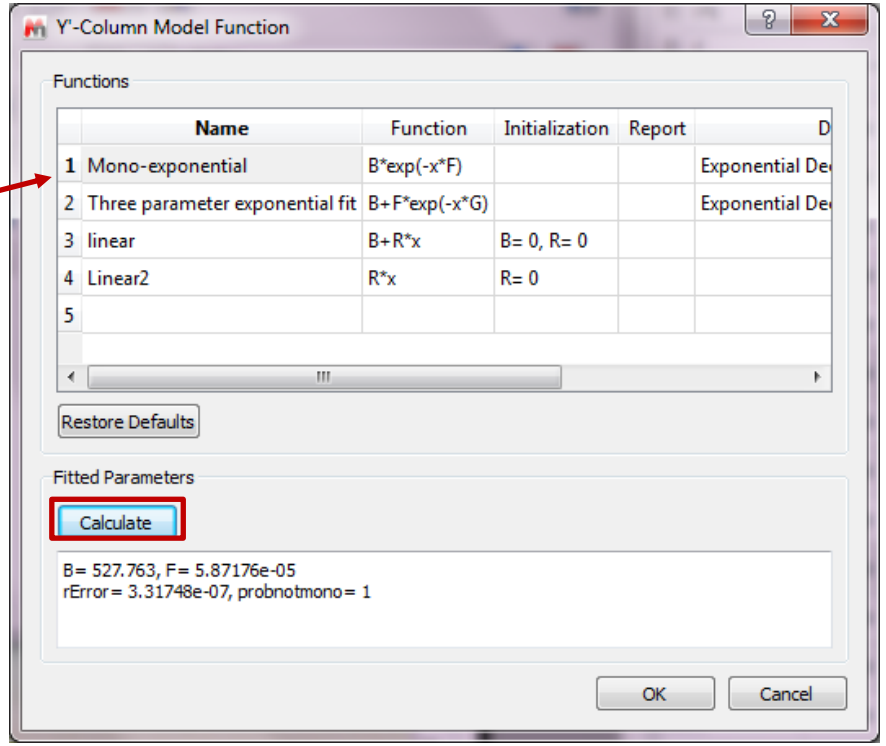
Click & drag the handles to exclude data points at either end of the data series

MESTRELAB RESEARCH
Chemistry Software Solutions

To fit the data to a function

- To fit the XY points to a function, double click the first cell in the Y'(X) column, and choose (or define) a function, and click **Calculate** to do the fitting. Click OK to accept the results:

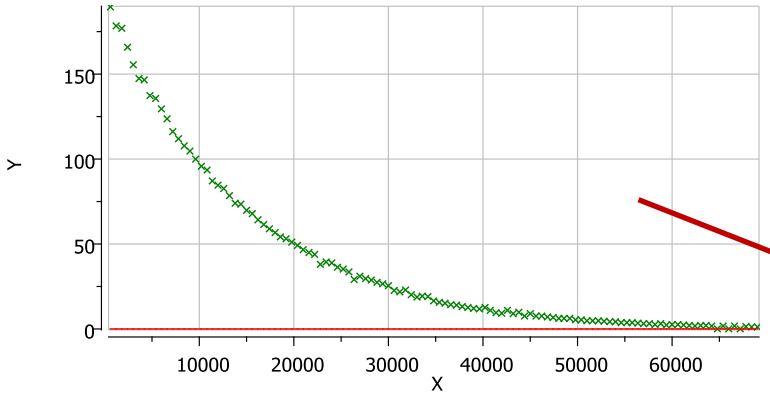


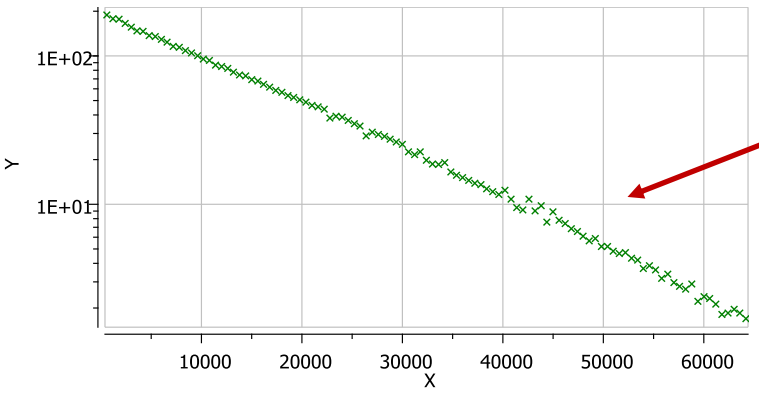


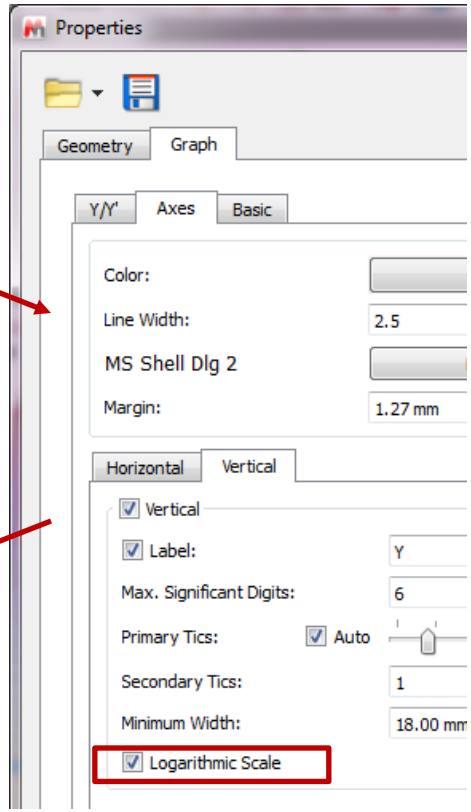
This example shows a first order reaction. F is the rate constant (k). The half-life $t_{1/2}$ = 0.693/F

To fit the data to a function

- To change the Y scale of the XY graph to logarithmic, right click on the graph and choose **Properties**, and toggle on the **Logarithmic Scale** option:



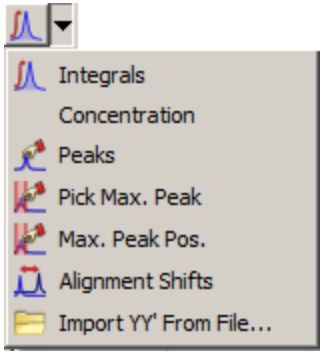


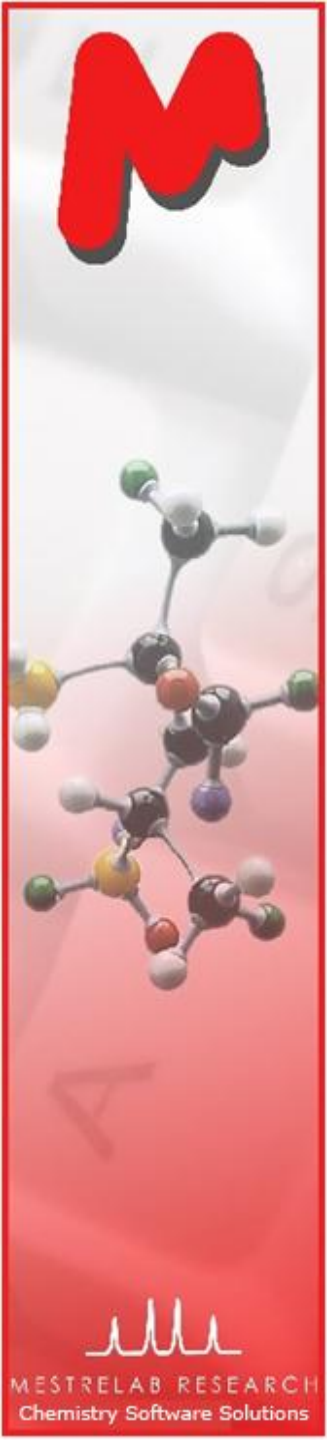


Tip: We recommend you to fit the original values to exponential function directly to avoid bigger numeric fitting errors. Also: Make sure you exclude zero or negative values before converting them to logarithmic scale.

More about the Data Analysis Panel

- You can click the "**+**" button to add another Y(X) column to the current table/plot, or you can start with a new table/plot by clicking the **Create New Plot** button.
- You can use other types of spectral properties as Y(X) values:
  - **Integrals**: peak integrals
  - **Concentration**: concentrations of an analyte
  - **Peaks**: intensities of the peaks near a defined location
  - **Maximum Peaks**: intensities of the highest peaks in a defined region
  - **Max. Peak Positions**: positions of the highest peaks in a defined region.
  - **Pick Alignment Shifts**: the shifts of peaks relative to the peak in the first spectrum





Summary

- Mnova NMR provides powerful and easy-to-use tools for processing and analysis of multiple NMR spectra for **reaction monitoring**
- Such tools can be used for many other types of studies, such as **relaxation, diffusion, molecular binding studies**, etc.
- See **Help | Contents | Advanced Menu | Data Analysis** for more info.
- For 45 day free trial of Mnova, go to http://mestrelab.com/software/mnova-suite/download/ or email us at sales@mestrelab.com